





Complete Genome Sequence of *Sinorhizobium meliloti* Strain AK21, a Salt-Tolerant Isolate from the Aral Sea Region

 José I. Jiménez-Zurdo,^a Francisco Martínez-Abarca,^a José F. Cobo-Díaz,^b José A. López-Contreras,^a
 Manuel Fernández-López,^a Nicolás Toro^a

^aStructure, Dynamics, and Function of Rhizobacterial Genomes Research Group, Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain

^bDepartamento de Higiene y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de León, León, Spain

ABSTRACT We report here the complete genome sequence of the salt-tolerant *Sinorhizobium meliloti* strain AK21, isolated from nodules of *Medicago sativa* L. subsp. *ambigua* inhabiting the northern Aral Sea Region. This genome (7.36 Mb) consists of a chromosome and four accessory plasmids, two of which are the symbiotic megaplasmids pSymA and pSymB.

The mutualistic symbioses established between certain species of the *Alphaproteobacteria* and *Betaproteobacteria* classes (collectively known as rhizobia) and legumes provide a great proportion of the N₂ in the biosphere (1). Symbiotic nitrogen fixation is compromised by the harsh conditions (e.g., drought or salinity) commonly affecting most agricultural soils (2, 3). Thus, management of legume varieties and rhizobial strains naturally adapted to abiotic stress may improve crop yields in unfavorable environments (4). The widespread interactions between the α -rhizobium *Sinorhizobium meliloti* and legumes of the *Medicago* genus are a model experimental system to investigate symbiosis (5–7). The two most recently published *S. meliloti* draft genome sequences correspond to strains AK170 and AK555, isolated from wild-growing *Medicago* plants in northwest Kazakhstan in the Aral Sea region (8–10). This area suffers from man-made pollution, drought, and salinization, thus constituting a source of salt-tolerant rhizobia with expected increased symbiotic competence under these extreme conditions (11).

We announce here the complete, fully assembled genome sequence of another member of this Aral Sea strain collection, *S. meliloti* AK21 (SmeAK21), isolated from the nodules of *Medicago sativa* L. subsp. *ambigua* (formerly named *Medicago trautvetteri*) plants (8). A previous prospective study already unveiled the possession of specific metabolic capabilities by SmeAK21 and large genomic differences with respect to the *S. meliloti* reference strain Rm1021 (12, 13). The total DNA was obtained with a Real genomic DNA purification kit (Durviz S.L.) from a pure SmeAK21 culture in complete tryptone yeast broth (14). Sequencing was performed on the GS FLX Titanium platform (Roche Diagnostics) at Macrogen, Inc. (South Korea), using both shotgun and 3-kb mate-paired libraries prepared according to Roche 454 standard protocols (15), which delivered totals of 1,607,710 (average length, 719 bp) and 520,432 (average length, 414 bp) reads, respectively. The quality (Phred) score was 40 or above for 99.98% of the bases. The raw data were processed using the Roche GS FLX software (v2.6) and the GS De Novo Assembler tool (v2.6), using default parameters. These analyses assembled the reads into 27 primary scaffolds (*N*₅₀ value, 587,174 bp; average length, 266,493 bp; longest scaffold, 1,524,556 bp), predicting 190-fold coverage of the genome. Intra- and interscaffold gaps were further closed by mapping and assembling the specific 3-kb mate-paired library reads to both borders of each gap using the tools of the Geneious Basic platform (16). When required, the Rm1021 genome was used as a reference (12).

Citation Jiménez-Zurdo JI, Martínez-Abarca F, Cobo-Díaz JF, López-Contreras JA, Fernández-López M, Toro N. 2020. Complete genome sequence of *Sinorhizobium meliloti* strain AK21, a salt-tolerant isolate from the Aral Sea region. Microbiol Resour Announc 9:e01432-19. <https://doi.org/10.1128/MRA.01432-19>.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2020 Jiménez-Zurdo et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to José I. Jiménez-Zurdo, jjz@eez.csic.es.

Received 18 November 2019

Accepted 12 December 2019

Published 9 January 2020

The complete genome consists of 7.36 Mb distributed into five replicons with the following features (size and G+C content, respectively): the main chromosome (3,785,735 bp and 62.7%), two symbiotic megaplasmids that are present in all *S. meliloti* strains, i.e., pSymA (1,506,823 bp and 55.7%) and pSymB (1,676,618 bp and 62.4%), and two smaller accessory plasmids that we have termed pSmeAK21a (151,735 bp and 61.5%) and pSmeAK21b (209,149 bp and 59.3%). All four plasmids have recognizable rhizobial *repABC* replication origins (17). Annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline, which predicted 6,810 protein-coding genes, 53 tRNA loci, and 3 rRNA operons.

This genome provides a new resource to investigate the adaptive mechanisms of rhizobia to abiotic stress and their potential exploitation in sustainable agricultural practices.

Data availability. The sequences of the SmeAK21 chromosome, pSymA, pSymB, pSmeAK21a, and pSmeAK21b have been deposited at GenBank under the accession numbers [CP026525](#), [CP026526](#), [CP026527](#), [CP026528](#), and [CP026529](#), respectively. The Sequence Read Archive (SRA) accession numbers for the raw data are [SRR10441808](#), [SRR10441809](#), and [SRR10441810](#). The BioProject accession number is [PRJNA432049](#).

ACKNOWLEDGMENTS

Strain SmeAK21 was provided by Marina Roumiantseva and Boris Simarov to the partners of the International Cooperation-Cooperation with the Countries of Central Europe and with the New Independent States of the Former Soviet Union (INCO-COPERNICUS) program (grant ICA2-CT-2001-10001), and it is available at the All-Russia Research Institute for Agricultural Microbiology (ARRIAM; strain collection number RCAM05222).

This work was funded by European Regional Development Fund (ERDF)-cofinanced grants from the Spanish Ministerio de Ciencia, Innovación y Universidades (grant CSD2009-00006 to N.T. and grants AGL2009-07925 and BFU2017-82645-P to J.I.J.-Z.).

REFERENCES

- Herridge DF, Peoples MB, Boddey RM. 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311:1–18. <https://doi.org/10.1007/s11104-008-9668-3>.
- Zahran HH. 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol Mol Biol Rev* 63:968–989.
- Karmakar K, Rana A, Rajwar A, Sahgal M, Johri BN. 2015. Legume-rhizobia symbiosis under stress, p 241–258. In Arora NK (ed), *Plant microbes symbiosis: applied facets*. Springer India, New Delhi, India. https://doi.org/10.1007/978-81-322-2068-8_12.
- Zahran HH. 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *J Biotechnol* 91:143–153. [https://doi.org/10.1016/S0168-1656\(01\)00342-X](https://doi.org/10.1016/S0168-1656(01)00342-X).
- Jiménez-Zurdo JI, Valverde C, Becker A. 2013. Insights into the noncoding RNome of nitrogen-fixing endosymbiotic α -proteobacteria. *Mol Plant Microbe Interact* 26:160–167. <https://doi.org/10.1094/MPMI-07-12-0186-CR>.
- Nelson M, Guhlín J, Epstein B, Tiffin P, Sadowsky MJ. 2018. The complete replicons of 16 Ensifer meliloti strains offer insights into intra- and inter-replicon gene transfer, transposon-associated loci, and repeat elements. *Microb Genom* 4. <https://doi.org/10.1099/mgen.0.000174>.
- Epstein B, Abou-Shanab RAI, Shamseldin A, Taylor MR, Guhlín J, Burghardt LT, Nelson M, Sadowsky MJ, Tiffin P. 2018. Genome-wide association analyses in the model rhizobium Ensifer meliloti. *mSphere* 3:e00386–18. <https://doi.org/10.1128/mSphere.00386-18>.
- Ibragimova MV, Rumyantseva ML, Onishchuk OP, Belova VS, Kurchak ON, Andronov EE, Dzyubenko NI, Simarov BV. 2006. Symbiosis between the root-nodule bacterium Sinorhizobium meliloti and alfalfa (*Medicago sativa*) under salinization conditions. *Microbiology* 75:77–81. <https://doi.org/10.1134/S0026261706010140>.
- Baturina OA, Muntyan VS, Cherkasova ME, Saksaganskaya AS, Dzyubenko NI, Kabilov MR, Roumiantseva ML. 2019. Draft genome sequence of Sinorhizobium meliloti strain AK170. *Microbiol Resour Announc* 8:e01571–18. <https://doi.org/10.1128/MRA.01571-18>.
- Muntyan VS, Baturina OA, Afonin AM, Cherkasova ME, Laktionov YV, Saksaganskaya AS, Kabilov MR, Roumiantseva ML. 2019. Draft genome sequence of Sinorhizobium meliloti AK555. *Microbiol Resour Announc* 8:e01567–18. <https://doi.org/10.1128/MRA.01567-18>.
- Roumiantseva ML, Muntyan VS. 2015. Root nodule bacteria Sinorhizobium meliloti: tolerance to salinity and bacterial genetic determinants. *Microbiology* 84:303–318. <https://doi.org/10.1134/S0026261715030170>.
- Galibert F, Finan TM, Long SR, Puhler A, Abola P, Ampe F, Barloy-Hubler F, Barnett MJ, Becker A, Boistard P, Bothe G, Boutry M, Bowser L, Buhrmester J, Cadieu E, Capela D, Chain P, Cowie A, Davis RW, Dreano S, Federspiel NA, Fisher RF, Gloux S, Godrie T, Goffeau A, Golding B, Guzy J, Gurjal M, Hernandez-Lucas I, Hong A, Huizar L, Hyman RW, Jones T, Kahn D, Kahn ML, Kalman S, Keating DH, Kiss E, Komp C, Lelaure V, Masuy D, Palm C, Peck MC, Pohl TM, Portetelle D, Purnelle B, Ramsperger U, Surzycki R, Thebault P, Vandenbol M, et al. 2001. The composite genome of the legume symbiont *Sinorhizobium meliloti*. *Science* 293:668–672. <https://doi.org/10.1126/science.1060966>.
- Molina-Sánchez MD, López-Contreras JA, Toro N, Fernández-López M. 2015. Genomic characterization of Sinorhizobium meliloti AK21, a wild isolate from the Aral Sea region. *Springerplus* 4:259. <https://doi.org/10.1186/s40064-015-1062-z>.
- Beringer JE. 1974. R factor transfer in *Rhizobium leguminosarum*. *J Gen Microbiol* 84:188–198. <https://doi.org/10.1099/00221287-84-1-188>.
- Zheng Z, Advani A, Meleforts O, Glavas S, Nordström H, Ye W, Engstrand L, Andersson AF. 2011. Titration-free 454 sequencing using Y adapters. *Nat Protoc* 6:1367–1376. <https://doi.org/10.1038/nprot.2011.369>.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>.
- Cevallos MA, Cervantes-Rivera R, Gutiérrez-Ríos RM. 2008. The repABC plasmid family. *Plasmid* 60:19–37. <https://doi.org/10.1016/j.plasmid.2008.03.001>.